

## BASIC RESEARCH

# The effects of pneumoperitoneum and controlled ventilation on peritoneal lymphatic bacterial clearance: experimental results in rats

Armando Angelo Casaroli,<sup>I</sup> Lycia M. J. Mimica,<sup>II</sup> Belchor Fontes,<sup>III</sup> Samir Rasslan<sup>IV</sup>

<sup>I</sup> Departamento de Cirurgia da Santa Casa de Misericórdia de São Paulo, São Paulo/SP, Brazil. <sup>II</sup> Disciplina de Microbiologia e Imunologia da Faculdade de Ciências Médicas da Santa Casa de São Paulo, São Paulo/SP, Brazil. <sup>III</sup> Laboratório de Investigação Médica (LIM-62), Disciplina de Cirurgia Geral do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo/SP, Brazil. <sup>IV</sup> Disciplinas de Cirurgia Geral e do Trauma do Departamento de Cirurgia da Faculdade de Medicina da Universidade de São Paulo, São Paulo/SP, Brazil.

**OBJECTIVE:** To evaluate the effect of pneumoperitoneum, both alone and in combination with controlled ventilation, on peritoneal lymphatic bacterial clearance using a rat bacterial peritonitis model.

**METHOD:** A total of 69 male Wistar rats were intraperitoneally inoculated with an *Escherichia coli* solution ( $10^9$  colony-forming units (cfu)/mL) and divided into three groups of 23 animals each: A (control group), B (pneumoperitoneum under 5 mmHg of constant pressure), and C (endotracheal intubation, controlled ventilation, and pneumoperitoneum as in Group B). The animals were sacrificed after 30 min under these conditions, and blood, mediastinal ganglia, lungs, peritoneum, liver, and spleen cultures were performed.

**RESULTS:** Statistical analyses comparing the number of cfu/sample in each of the cultures showed that no differences existed between the three groups.

**CONCLUSION:** Based on our results, we concluded that pneumoperitoneum, either alone or in association with mechanical ventilation, did not modify the bacterial clearance through the diaphragmatic lymphatic system of the peritoneal cavity.

**KEYWORDS:** Diaphragm; Pneumoperitoneum; Mechanical Ventilation; Peritoneal Infection; Rats.

Casaroli AA, Mimica LMJ, Fontes B, Rasslan S. The effects of pneumoperitoneum and controlled ventilation on peritoneal lymphatic bacterial clearance: experimental results in rats. *Clinics*. 2011;66(9):1621-1625.

Received for publication on March 16, 2011; First review completed on April 15, 2011; Accepted for publication on June 2, 2011

E-mail: armandangelo@uol.com.br

Tel.: 55 11 30696555

## INTRODUCTION

The use of video-laparoscopy has significantly contributed to urgent abdominal surgery, both as a diagnostic method in acute cases and as an alternative, and even preferred, procedure for treating certain diseases.<sup>1,2</sup> For many years, the presence of peritonitis was considered to be a significant risk factor for laparoscopy. However, research examining the use of video-laparoscopy in the presence of peritoneal infection has reported favorable clinical results.<sup>2,3</sup>

The presence of bacteria in the peritoneal cavity and its septic consequences have motivated recent research.<sup>4</sup> Historically, the concept that bacterial clearance by the diaphragm is a negative rather than a positive prognostic factor was first suggested by the reduced mortality of peritonitis patients who were maintained in a semi-seated position that decreases diaphragmatic contact with peritoneal

secretions.<sup>5</sup> Although this concept has faced opposition,<sup>6</sup> it is supported by studies reporting improved survival in animal peritonitis models following procedures designed to prevent bacterial clearance, such as scarification of the diaphragmatic surface and using substances to block the diaphragmatic pores.<sup>7</sup> Other studies have observed that carbon particles injected into the mouse peritoneum are found in Kupffer cells 20 min later, possibly after absorption by the diaphragm and migration through blood and lymphatic transport systems.<sup>8</sup> Thus, the possible role of the diaphragm in this process has remained unclear. More recent studies have shown that a CO<sub>2</sub> insufflation-induced pneumoperitoneum increases bacterial (*E. coli*) translocation in rats,<sup>9</sup> and others have observed that a CO<sub>2</sub> pneumoperitoneum is associated with positive-end expiratory pressure and has neither a positive nor a negative impact on the systemic expansion of intra-abdominal *E. coli* infection.<sup>10</sup>

Despite frequent and progressive clinical utilization of video-laparoscopy in urgent abdominal surgery, certain questions concerning the possible effects of pneumoperitoneum on patients with abdominal infections remain unanswered. It has been argued that the increase in abdominal pressure induced by the pneumoperitoneum

could promote greater absorption of bacteria and toxins from the peritoneal cavity, which could increase the risk of septic shock if they enter the bloodstream.<sup>11</sup>

The distension of the peritoneal cavity produced by pneumoperitoneum causes alterations in diaphragmatic movements, intra-abdominal and intra-thoracic pressures, and respiratory dynamics, and these factors are usually considered to be involved in lymphatic drainage of the peritoneum. However, the true effect of pneumoperitoneum on the diaphragmatic lymphatic drainage system remains unclear. Similarly, it is not known whether the combination of controlled ventilation and constant-pressure pneumoperitoneum, which is commonly employed in video-laparoscopic surgeries, increases or decreases the removal of substances from the peritoneal cavity. In many experimental animal studies involving sepsis and pneumoperitoneum, controversy concerning the repercussions of video-surgery in the presence of intra-abdominal infection remains.<sup>12-15</sup>

Thus, the objective of this study was to use an experimental rat peritoneal contamination model to evaluate the influence of pneumoperitoneum, both alone and in combination with controlled ventilation, on lymphatic bacterial clearance from the peritoneal cavity.

## MATERIALS AND METHODS

A total of 69 adult male Wistar rats, weighing 200-300 g, was maintained in the laboratory on *ad libitum* water and standard diet for less than seven days. They were randomly divided into three groups of 23 rats each. All of the animals were anesthetized using intramuscular injections of ketamine (100 mg/kg) and xylazine (10 mg/kg) in the medial face of the thigh, and a 0.5-cm incision was made beneath the umbilical cicatrix to expose the delicate aponeurotic muscle layer. While light traction was maintained on the abdominal wall, a peritoneal puncture was performed with an 18-G Teflon catheter-embedded needle. After removing the metallic needle, the peritoneal cavity was inoculated with 1 mL of 10<sup>9</sup> colony forming units (cfu)/mL *E. coli* (ATCC pattern) solution. The Teflon catheter was maintained in all of the groups, with and without pneumoperitoneum, until the end of the experiment.

After anesthesia and asepsis, the Group A rats were maintained under spontaneous respiration for a period of 30 min, the Group B rats were maintained under spontaneous respiration and pneumoperitoneum for a period of 30 min, and the Group C rats were intubated and maintained under controlled respiration and pneumoperitoneum for a period of 30 min. Instead of the usual tracheotomy, the orotracheal intubation in Group C used a technique to produce airways in small animals that was developed specifically for this experiment. To induce less surgical trauma, endotracheal access was obtained by direct laryngoscopy rather than a usual tracheotomy. The laryngoscopy used an optical video-laparoscope with a 5-mm diameter and a 30° angulated lens. The video-laparoscope was connected to a micro-camera system, light fountain, and video monitor. The anesthetized animal was maintained in the dorsal decubitus position, and its snout was lightly tractioned and lifted to create a space to introduce the optics. The optical system was used to correctly orient a number six Levine tube with a 5-cm extension in the transglottic position. Following the endotracheal intubation, the animals were maintained on mechanical ventilation

using a standard small-animal fan. The rats in Group C were maintained under controlled respiration with a minute volume of 400 mL and an average respiratory frequency of 40 incursions per minute.

The pneumoperitoneum was created after the bacterial inoculation while the rats remained under anesthesia. Animal electronic insufflators were used to distend the peritoneal cavity at a constant 5-mmHg pressure and a flux of 0.2-0.5 mL/min.

All of the groups were observed for 30 min. The pneumoperitoneum was then interrupted in Groups B and C, and the animals were sacrificed using a lethal dose of anesthesia.

Blood cultures were grown on a hemolisobac (PROBAC) medium, and organ tissue cultures were grown on cysteine lactose electrolyte-deficient Agar (CLED-Agar).

## Statistical analysis

The presence or absence of bacteria in the blood samples was expressed as the percentage of animals with positive cultures in each group, and the results were analyzed using the likelihood-ratio test. The Kruskal-Wallis test and an analysis of variance (ANOVA) model were used to compare the mean number of cfu in the cultures per gram of tissue collected between the groups. Statistical significance was set at  $p < 0.05$ .

## RESULTS

The blood culture measurements were expressed as cfu/mL of blood and analyzed as the percentage (%) of animals with positive cultures in each group. The other culture results were expressed as the number of cfu/g of tissue collected (Table 1).

No significant differences between Groups A, B, and C were found for any of the cultures (Table 1 and Figures 1, 2, and 3).

## DISCUSSION

In the context of video-laparoscopic surgery, the role of CO<sub>2</sub> insufflation-induced pneumoperitoneum in combination with mechanical ventilation in the dissemination of peritoneal bacterial infection and sepsis remains controversial.<sup>5,16,17</sup> When bacterial contamination is introduced into the peritoneal cavity, three major defense mechanisms are activated to remove the infection: bacterial clearance by the diaphragmatic lymphatic system, phagocytosis by local macrophages, and migration of neutrophils to the abdomen. Lymphatic drainage and macrophage activity are the first lines of defense against bacteria following peritoneal contamination.<sup>5,18,19</sup>

Due to its considerable level of communication with the rich lymphatic system, the diaphragmatic peritoneum confers the particular function of bacterial clearance from the peritoneum upon the diaphragm. Bacteria are removed from the peritoneal cavity through this lymphatic system and reach the mediastinal lymph nodes through the retrosternal nodes.<sup>20,21</sup> A decrease in diaphragmatic mobility causes a decline in bacterial clearance from the peritoneum.<sup>22,23</sup> Thus, the dampening effect of mechanical ventilation on diaphragmatic dynamics could be expected to attenuate this bacterial clearance mechanism.<sup>19</sup> Another factor that interferes with the absorption of substances by

**Table 1** - Comparison of the blood (qualitative) and other tissue (quantitative) culture results between the three groups.

| Animal groups<br>All animals were bacterially inoculated.<br>(n = 23) |  |   |  | Comparison of the<br>three groups |
|---|--|---|--|-----------------------------------|
|   | No pneumoperitoneum                            | With pneumoperitoneum                         | Pneumoperitoneum +<br>Mechanical ventilation | p-value                           |
| Diaphragm<br>Mean (SD)  | 42.0×10 <sup>5</sup> (53.3×10 <sup>5</sup> )   | 48.4×10 <sup>5</sup> (66.5×10 <sup>5</sup> )  | 25.2×10 <sup>5</sup> (35.8×10 <sup>5</sup> ) | 0.4379*                           |
| Mediastinal lymph nodes<br>Mean (SD)                                  | 141.3×10 <sup>5</sup> (476.4×10 <sup>5</sup> ) | 38.1×10 <sup>5</sup> (77.5×10 <sup>5</sup> )  | 20.2×10 <sup>5</sup> (51.9×10 <sup>5</sup> ) | 0.8254*                           |
| Liver<br>Mean (SD)  | 12.0×10 <sup>5</sup> (27.9×10 <sup>5</sup> )   | 12.9×10 <sup>5</sup> (21.1×10 <sup>5</sup> )  | 21.2×10 <sup>5</sup> (40.0×10 <sup>5</sup> ) | 0.5405**                          |
| Spleen<br>Mean (SD)   | 10.8×10 <sup>5</sup> (25.3×10 <sup>5</sup> )   | 31.8×10 <sup>5</sup> (61.3×10 <sup>5</sup> )  | 17.6×10 <sup>5</sup> (35.9×10 <sup>5</sup> ) | 0.2561**                          |
| Peritoneum<br>Mean (SD)   | 51.8×10 <sup>5</sup> (79.7×10 <sup>5</sup> )   | 78.7×10 <sup>5</sup> (120.7×10 <sup>5</sup> ) | 32.8×10 <sup>5</sup> (49.9×10 <sup>5</sup> ) | 0.1841*                           |
| Lung<br>Mean (SD)   | 6.6×10 <sup>5</sup> (16.0×10 <sup>5</sup> )    | 3.8×10 <sup>5</sup> (7.8×10 <sup>5</sup> )    | 5.5×10 <sup>5</sup> (16.5×10 <sup>5</sup> )  | 0.8049**                          |
| Blood<br>n (%)  | 7 (30.4%)                                      | 8 (34.8%)                                     | 7 (30.4%)                                    | 0.9354***                         |
| 95% CI  | [19.44%; 68.06%]                               | [28.09%; 78.58%]                              | [19.44%; 68.06%]                             |                                   |

CI - Confidence interval.

\*p-value for the nonparametric Kruskal-Wallis test.

\*\*p-value for the ANOVA model.

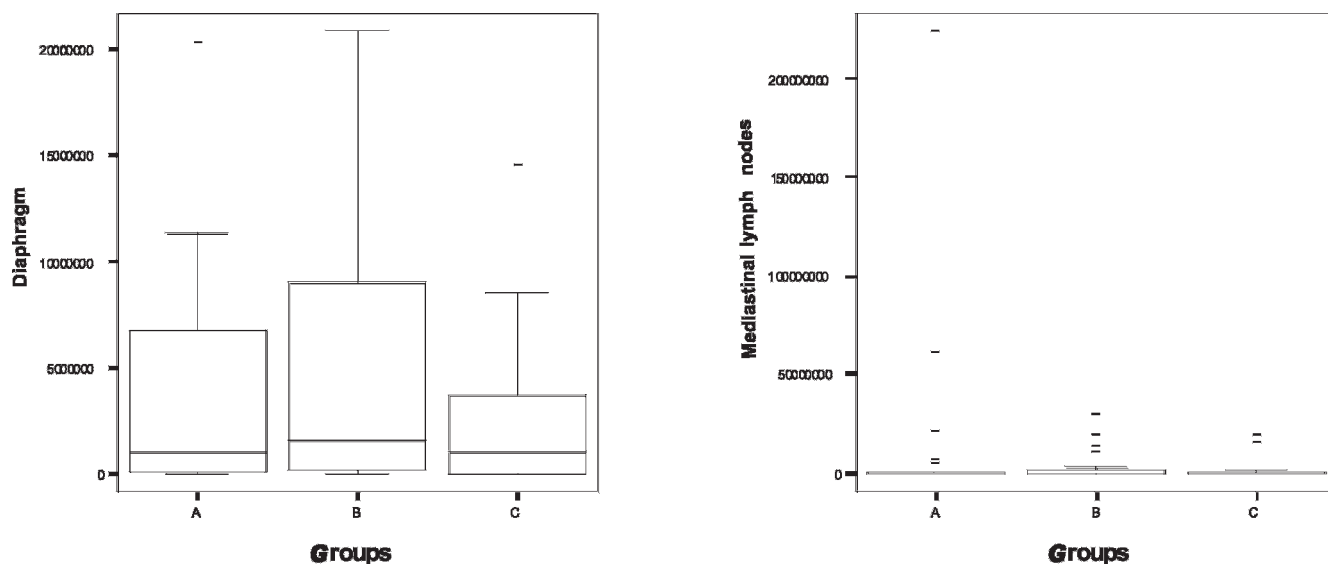
\*\*\*p-value for the likelihood ratio test.

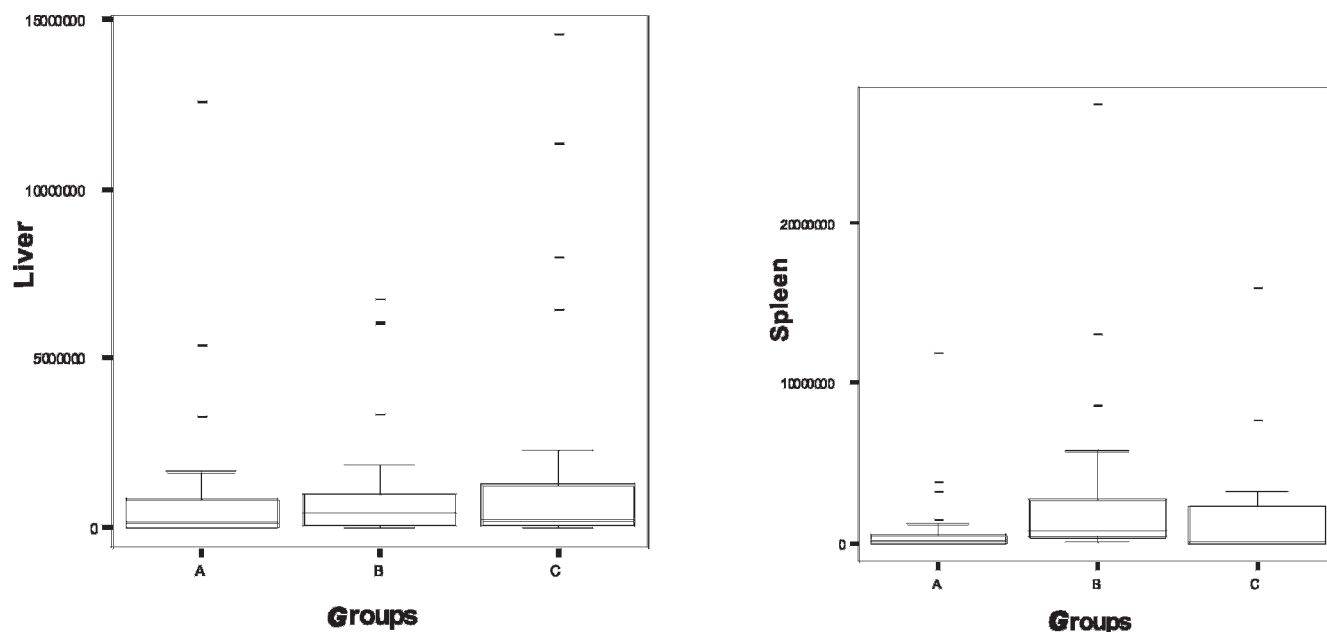
There were no significant differences between the groups for any of the variables in the Table.

the diaphragmatic lymphatic system is abdominal pressure; there is a direct correlation between abdominal pressure and diaphragmatic lymphatic bacterial clearance.<sup>24,25</sup> The gaseous pneumoperitoneum used in laparoscopic surgery causes elevated intra-abdominal pressure and leads to increased diaphragmatic bacterial clearance from the peritoneal cavity. It also leads to peritoneal distension, which promotes diaphragmatic lengthening and interferes with diaphragmatic movement. This condition may reduce bacterial clearance from the peritoneal cavity.<sup>26</sup> However,

the combined effect of these two antagonistic factors on diaphragm-mediated bacterial removal remains unclear.

In this context, we hypothesized that these two opposing factors compete to define the lymphatic bacterial clearance from the peritoneal cavity. On the one hand, the pneumoperitoneum increases the intra-abdominal pressure, which favors bacterial clearance via the lymphatic system. On the other hand, lengthening of the diaphragmatic surface and controlled respiration decrease the lymphatic pumping action of the diaphragm,<sup>22</sup> which results in reduced

**Figure 1** - Box-plots for diaphragmatic and mediastinal lymph node tissue cultures. Groups: A=no pneumoperitoneum, B=pneumoperitoneum, and C=pneumoperitoneum + mechanical ventilation.



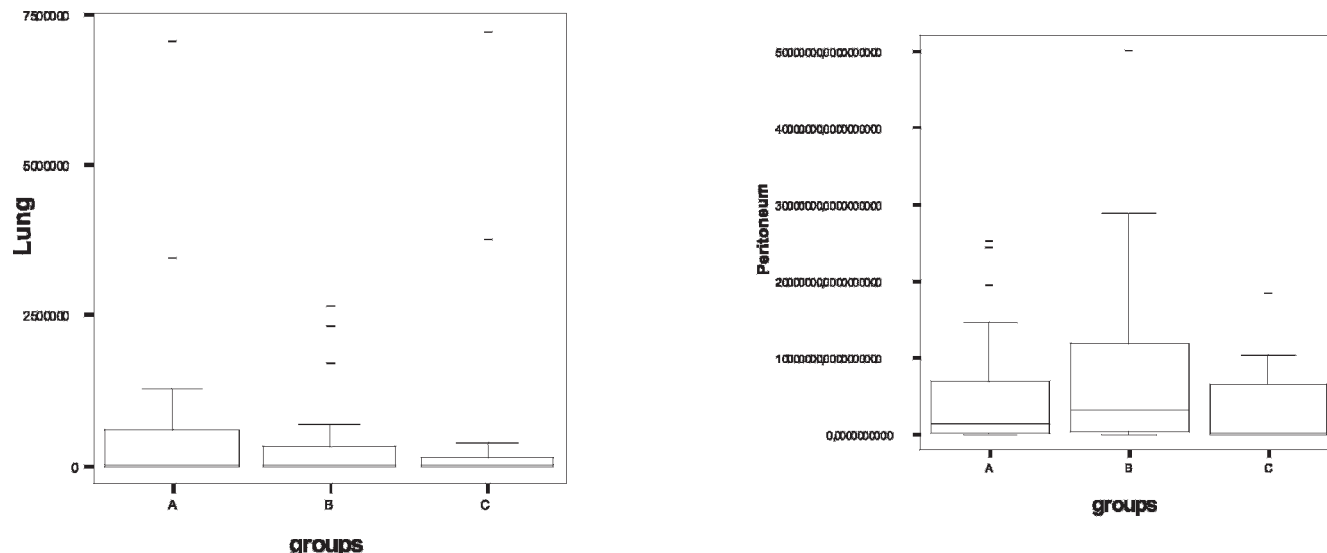
**Figure 2** - Box-plots for liver and spleen tissue cultures. Groups: A = no pneumoperitoneum, B = pneumoperitoneum, and C = pneumoperitoneum + mechanical ventilation.

bacterial clearance. The 30-min period used in this study was based on previous experiments showing that peritoneal lymphatic clearance of particulate matter is quickly initiated, becoming detectable in minutes, and absorbs most of the material in approximately 30 min.<sup>27,28</sup> Thus, controlled ventilation combined with pneumoperitoneum was used to simulate the conditions of laparoscopic surgical procedures that employ pneumoperitoneum and mechanical ventilation.

We found no significant differences between the three groups based on blood, liver, lung, spleen, and peritoneum cultures. We also did not observe significant differences

between the groups in the numbers of *E. coli* cfu in the diaphragmatic or mediastinal lymph nodes, which participate in the most relevant mechanism for bacterial clearance.

The similarities between the three groups in this study may have resulted from complex, synergistic effects of the pneumoperitoneum and mechanical ventilation on the diaphragmatic lymphatic system, as discussed above. Furthermore, no significant differences between the three groups were observed in our hemoculture results. Similar hemoculture results have been observed in other studies.<sup>28,29</sup> Additionally, no evidence of hemodynamic alterations or increased bacteremia or endotoxemia have been



**Figure 3** - Box-Plots for Peritoneum and Lung tissue cultures. Groups: A=no pneumoperitoneum, B=pneumoperitoneum, and C=pneumoperitoneum + mechanical ventilation.

reported in several other studies that examined the effect of pneumoperitoneum on the dissemination of peritoneal bacteria in animal models.<sup>12,29,30</sup> However, other studies have reported an increased incidence of bacteremia one hour after insufflation of the peritoneal cavity.<sup>31,32</sup> Overall, no clear consensus can be obtained from the results of experiments that have investigated this issue.

## CONCLUSION

Based on our results, we conclude that pneumoperitoneum, either alone or in combination with mechanical ventilation, does not modify the lymphatic clearance of peritoneal bacteria through diaphragmatic drainage. More studies are required to clarify the conflicting results observed in the literature on this topic.

## REFERENCES

- Salky BA, Edey MB. The role of laparoscopy in the diagnosis and treatment of abdominal pain syndromes. *Surg Endosc*. 1998;12:911-4, doi: 10.1007/s004649900744.
- Navez B, Tasseti V, Scohy JJ, Mutter D, Guiot P. Laparoscopic Management of acute peritonitis. *Br J Surg*. 1998;85:32-6, doi: 10.1046/j.1365-2168.1998.00531.x.
- Geis WP, Kim HC. Use of laparoscopy in the diagnosis and treatment of patients with surgical abdominal sepsis. *Surg Endosc*. 1995;9:178-82, doi: 10.1007/BF00191962.
- Souza YM, Fontes B, Martins JO, Sannomiya P, Brito GS, Younes RN, Rasslan S. Evaluation of the effects of ozone therapy in the treatment of intra-abdominal infection in rats. *Clinics*. 2010;65:195-202, doi: 10.1590/S1807-59322010000200012.
- Maddaus MA, Ahrenholz D, Simmons R. The biology of peritonitis and implications for treatment. *Surg Clin North Am*. 1988; 68:431-443.
- Steinberg B. Infections of the Peritoneum. New York, Paul Hoeber Inc, 1944;455.
- Silva LN, Cardoso MB, Gondek JFL, Esmanhotto LB, Sebastião APM, Simões JC. Peritonite aguda experimental em ratos modelo de bloqueio transdiafragmático com membrana celulósica. *Rev Col Bras Cir*. 1998;25:113-7, doi: 10.1590/S0100-69911998000200008.
- Abu-Hijleh MF, Habbal OA, Moqattash ST. The role of the diaphragm in lymphatic absorption from the peritoneal cavity. *J Anat*. 1995;186:453-67.
- Horattas MC, Haller N, Ricchiuti D. Increased transperitoneal bacterial Translocation in laparoscopic surgery. *Surg Endosc*. 2003;17:1464-7, doi: 10.1007/s00464-001-8289-1.
- Barbaros U, Ozarmagan S, Erbil Y, Bozbora A, Cakar N, Eraksoy H, Kapran Y, Kiran B. Effects of pneumoperitoneum created through CO<sub>2</sub> insufflation and parameters of mechanical ventilation (PEEP application) on systemic dissemination of intraabdominal infections. *Surg Endosc*. 2004;8:501-7, doi: 10.1007/s00464-003-9107-8.
- Greif WM, Forse RA. Hemodynamic effects of the laparoscopic pneumoperitoneum during sepsis in a porcine endotoxemic shock model. *Ann Surg*. 1998;227:474-80, doi: 10.1097/0000658-199804000-00004.
- Collet D, Vitale GC, Reynolds M, Klar E, Cheadle WG. Peritoneal host defenses are less impaired by laparoscopy than by open operation. *Surg Endosc*. 1995;9:1059-64, doi: 10.1007/BF00188987.
- Bustos B, Gómez-Ferrer F, Balique JG, Porcheron J, Gobernado M, Cantón E. Laparoscopy and septic dissemination caused by perioperative perforation of the occluded small bowel: an experimental study. *Surg Laparosc Endosc*. 1997;7:228-31, doi: 10.1097/00019509-199706000-00010.
- Dugue L, Fritsch S, Felten A, Gossot D, Colomer S, Celerier M, et al. Effects of intraperitoneal insufflation on hematogenous seeding of abdominal infections: Preliminary results of an experimental study in rats. *Ann Chir*. 1995;49:423-6.
- Jacobi CA, Ordemann J, Bohm B, Zieren HU, Volk HD, Lorenz W, et al. Does laparoscopy increase bacteremia and endotoxemia in a peritonitis model? *Surg Endosc*. 1997;11:235-8, doi: 10.1007/s004649900333.
- Balagué PC, Trias M. Laparoscopic surgery and surgical infection. *J Chemother*. 2001;13 Spec. 1:17-22.
- Targarona EM, Rodríguez M, Camacho M, Balagué C, Gich I, Vila L, et al. Immediate peritoneal response to bacterial contamination during laparoscopic surgery. *Surg Endosc*. 2006;20:316-21, doi: 10.1007/s00464-005-0367-3.
- Leak LV. Interaction of mesothelium to intraperitoneal stimulation. I. Aggregation of peritoneal cells. *Lab Invest*. 1983;48:479-91.
- Skau T, Nyström PO, Ohman L, Stendahl O. Bacterial clearance and granulocyte response in experimental peritonitis. *J Surg Res*. 1986;40:13-20, doi: 10.1016/0022-4804(86)90139-3.
- Tsilibary EC, Wissig SL. Light and electron microscope observation of the lymphatic drainage units of the peritoneal cavity of rodents. *Am J Anat*. 1987;180:195-207, doi: 10.1002/aja.1001800209.
- Abu-Hijleh MF, Scothorne RJ. Regional Lymph Drainage Routes From the Diaphragm in the Rat. *Clin Anat*. 1994;7:181-8, doi: 10.1002/ca.980070403.
- Bettendorf U. Lymph flow mechanism of the subperitoneal diaphragmatic lymphatics. *Lymphology*. 1978;11:111-6.
- Mengle HA. Effect of anaesthetics on lymphatic absorption from the peritoneal cavity in peritonitis: an experimental study. *Arch Surg*. 1937;34:39-52.
- Forey H. Reactions of, and absorption by lymphatics, with special reference to those of the diaphragm. *Br J Exp Pathol*. 1927;28:479-90.
- Zink J, Greenway CV. Control of ascites absorption in anesthetized cats; effects of intraperitoneal pressure, protein and furosemide diuresis. *Gastroenterology*. 1977;73:1119-24.
- SareM, YesiladaO, GürelM, BalkayaM, YologluS, FiskinK. Effects of CO<sub>2</sub> insufflation on bacterial growth in rats with *Escherichia coli*-induced experimental peritonitis. *Surg Laparosc Endosc*. 1997;7:38-41, doi: 10.1097/00019509-199702000-00010.
- Courtice FC, Steinbeck AW. The lymphatic drainage of plasma from the peritoneal cavity of the cat. *Aust J Exp Biol Med Sci*. 1950;28:161-9, doi: 10.1038/icb.1950.15.
- Hau T, Simmons RL. Mechanisms of the adjuvant effect of hemoglobin in experimental peritonitis. III. The influence of hemoglobin on phagocytosis and intracellular killing by human granulocytes. *Surgery*. 1980;87:588-92.
- Gurtner GC, Robertson CS, Chung CS, Ling TK, Ip SM, Li AK. Effect of carbon dioxide pneumoperitoneum on bacteraemia and endotoxaemia in an animal model of peritonitis. *Br J Surg*. 1995;82:844-8, doi: 10.1002/bjs.1800820639.
- Silva FCS. Análise da concentração sanguínea de bactérias durante insuflação peritoneal com dióxido de carbono em cães com peritonite bacteriana. São Paulo, 1997 (Doctorate thesis, University of São Paulo School of Medicine).
- Ipek TM, Paksoy T, Colak E, Polat E, Uygun N. Effect of carbon dioxide pneumoperitoneum on bacteremia and severity of peritonitis in an experimental model. *Surg Endosc*. 1998;12:432-5, doi: 10.1007/s004649900697.
- Jacobi CA, Ordemann J, Zieren HU, Volk HD, Bauhofer A, Halle E, et al. Increased Systemic Inflammation After Laparotomy vs Laparoscopy in an Animal Model of Peritonitis. *Arch Surg*. 1998;133:258-62, doi: 10.1001/archsurg.133.3.258.